UNIT 2.2 Preparation of Genomic DNA from Mammalian Tissue

BASIC PROTOCOL: PREPARATION OF GENOMIC DNA FROM MAMMALIAN TISSUE

Tissue is rapidly frozen and crushed to produce readily digestible pieces. The processed tissue is placed in a solution of proteinase K and SDS and incubated until most of the cellular protein is degraded. The digest is deproteinized by successive phenol/chloroform/isoamyl alcohol extractions, recovered by ethanol precipitation, and dried and resuspended in buffer.

Materials

Tissues, whole or cultured cells
Liquid nitrogen
Digestion buffer (see recipe)
PBS (*APPENDIX 2*), ice cold
7.5 M ammonium acetate
70% and 100% ethanol
TE buffer, pH 8 (*APPENDIX 2*)
Incubator or water bath at 50°C, with shaker

Additional reagents and equipment for trypsinizing adherent cells (*APPENDIX 3F*) and phenol/chloroform/isoamyl alcohol extraction (*UNIT 2.1A*)

Prepare cells

Beginning with whole tissue:

1a. As soon as possible after excision, quickly mince tissue and freeze in liquid nitrogen.

If working with liver, remove the gallbladder, which contains high levels of degradative enzymes.

2a. Starting with between 200 mg and 1 g, grind tissue with a prechilled mortar and pestle, or crush with a hammer to a fine powder (keep the tissue fragments, if crushing is incomplete).

3a. Suspend the powdered tissue in 1.2 ml digestion buffer per 100 mg tissue. There should be no clumps.

Beginning with tissue culture cells:

1b. Pellet suspension culture out of its serum-containing medium. Trypsinize adherent cells and collect cells from the flask. Centrifuge 5 min at 500 × *g*, 4°C, and discard supernatant.

2b. Resuspend cells with 1 to 10 ml ice-cold PBS. Centrifuge 5 min at 500 × *g* and discard supernatant. Repeat this resuspension and centrifugation step.

3b. Resuspend cells in 1 vol digestion buffer. For $<3 \times 10^7$ cells, use 0.3 ml digestion buffer. For larger numbers of cells use 1 ml digestion buffer/10^8 cells.

Lyse and digest cells

4. Incubate the samples with shaking at 50°C for 12 to 18 hr in tightly capped tubes.

The samples will be viscous. After 12 hr incubation the tissue should be almost indiscernible, a sludge should be apparent from the organ samples, and tissue culture cells should be relatively clear.

Extract nucleic acids

5. Thoroughly extract the samples with an equal volume of phenol/chloroform/isoamyl alcohol.

CAUTION: Phenol is extremely caustic.

6. Centrifuge 10 min at 1700 × *g* in a swinging bucket rotor.

If the phases do not resolve well, add another volume of digestion buffer, omitting proteinase K, and repeat the centrifugation.

If there is a thick layer of white material at the interface between the phases, repeat the organic extraction.

Purify DNA

7. Transfer the aqueous (top) layer to a new tube and add 1/2 vol of 7.5 M ammonium acetate and 2 vol (of original amount of top layer) of 100% ethanol. The DNA should immediately form a stringy precipitate. Recover DNA by centrifugation at 1700 × *g* for 2 min.

This brief precipitation in the presence of high salt

reduces the amount of RNA in the DNA. For long-term storage it is convenient to leave the DNA in the presence of ethanol.

Alternatively, to prevent shearing of high-molecular-weight DNA, omit steps 7 to 9 and remove organic solvents and salt from the DNA by at least two dialysis steps against at least 100 vol TE buffer. Because of the high viscosity of the DNA, it is necessary to dialyze for a total of at least 24 hr.

8. Rinse the pellet with 70% ethanol. Decant ethanol and air dry the pellet.

It is important to rinse well to remove residual salt and phenol.

9. Resuspend DNA at ~1 mg/ml in TE buffer until dissolved. Shake gently at room temperature or at 65°C for several hours to facilitate solubilization. Store indefinitely at 4°C.

From 1 g mammalian cells, ~2 mg DNA can be expected.

If necessary, residual RNA can be removed at this step by adding 0.1% sodium dodecyl sulfate (SDS) and 1 ug/ml DNase-free RNase (UNIT 3.13) and incubating 1 hr at 37°C, followed by organic extraction and ethanol precipitation, as above.

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REAGENTS AND SOLUTIONS

Note

Use deionized, distilled water in all recipes and protocol steps. For common stock solutions, see ***APPENDIX 2****; for suppliers, see* ***APPENDIX 4****.*

Digestion buffer

100 mM NaCl
10 mM Tris·Cl, pH 8 (*APPENDIX 2*)
25 mM EDTA, pH 8 (*APPENDIX 2*)
0.5% SDS
0.1 mg/ml proteinase K
Store at room temperature

The proteinase K is labile and must be added fresh with each use.

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0.1 mg/ml proteinase K
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APPENDIX 2 COMMONLY USED REAGENTS AND EQUIPMENT

BUFFERS AND STOCK SOLUTIONS

Introduction

This collection describes the preparation of buffers and reagents used in the manipulation of nucleic acids and proteins (see Table A.2.1). When preparing solutions, use deionized, distilled water and reagents of the highest grade available. Sterilization—by filtration through a 0.22-um filter or by autoclaving—is recommended for most applications. Recipes for the following can be found elsewhere in the manual: culture media (*UNIT 1.1*), antibiotics (Table 1.4.1), lactose analogs (Table 1.4.2), and enzyme buffers (*UNIT 3.4*).

CAUTION: *Handle strong acids and bases carefully.*

Acid precipitation solution

1 M HCl
0.1 M sodium pyrophosphate

Nucleic acids can also be precipitated with a 10% (w/v) solution of trichloroacetic acid (TCA); however, this recipe is cheaper, easier to prepare, and just as efficient.

Ammonium acetate, 10 M

Dissolve 385.4 g ammonium acetate in 150 ml H_2O
Add H_2O to 500 ml

BBS (BES-buffered solution), 2×

50 mM *N,N*-bis(2-hydroxyethyl)-2-aminoethanesulfonic acid (BES; Calbiochem)
280 mM NaCl
1.5 mM Na_2HPO_4, pH 6.95
800 ml H_2O
Adjust pH to 6.95 with room temperature 1 N NaOH

H_2O to 1 liter
Filter sterilize through a 0.45-um nitrocellulose filter (Nalgene)
Store in aliquots at -20°C (can be frozen and thawed repeatedly)

The pH of this solution is critical (pH 6.95 to 6.98). When a new batch of 2× BES buffer is prepared, its pH should be checked against a reference stock prepared (and tested) earlier.

$CaCl_2$, 1 M

147 g $CaCl_2 \cdot 2H_2O$
H_2O to 1 liter

Denhardt solution, 100×

10 g Ficoll 400
10 g polyvinylpyrrolidone
10 g bovine serum albumin (Pentax Fraction V; Miles Laboratories)
H_2O to 500 ml

Filter sterilize and store at -20°C in 25-ml aliquots

Dithiothreitol (DTT), 1 M

Dissolve 15.45 g DTT in 100 ml H_2O

Store at -20°C

EDTA (ethylenediamine tetraacetic acid), 0.5 M (pH 8.0)

Dissolve 186.1 g $Na_2EDTA \cdot 2H_2O$ in 700 ml H_2O
Adjust pH to 8.0 with 10 M NaOH (~50 ml)
Add H_2O to 1 liter

Ethidium bromide, 10 mg/ml

Dissolve 0.2 g ethidium bromide in 20 ml H_2O

Mix well and store at 4°C in dark

CAUTION: Ethidium bromide is a mutagen and must be handled carefully.

HBSS (Hanks balanced salt solution)

5.4 mM KCl
0.3 mM Na_2HPO_4
0.4 mM KH_2PO_4
4.2 mM $NaHCO_3$
1.3 mM $CaCl_2$

0.5 mM $MgCl_2$
0.6 mM $MgSO_4$
137 mM NaCl
5.6 mM D-glucose
0.02% phenol red (optional)
Add H_2O to l liter and adjust pH to 7.4

HBSS can be purchased from Biofluids or Whittaker.

HBSS may be made or purchased without $CaCl_2$ and $MgCl_2$. These are optional components that usually have no effect on an experiment. In some cases, however, their presence may be detrimental to a procedure. Consult the individual protocol to see if the presence or absence of these components is recommended in the materials list.

HCl, 1 M

Mix in the following order:
913.8 ml H_2O
86.2 ml concentrated HCl

HeBS (HEPES-buffered saline) solution, 2×

16.4 g NaCl
11.9 g HEPES acid
0.21 g Na_2HPO_4
800 ml H_2O
Titrate to pH 7.05 with 5 N NaOH
Add H_2O to 1 liter
Filter sterilize through a 0.45-um nitrocellulose filter
Test for transfection efficiency and store at -20°C in 50-ml aliquots

An exact pH is extremely important for efficient transfection. The optimal pH range is 7.05 to 7.12.

KCl, 1 M

74.6 g KCl
H_2O to 1 liter

$MgCl_2$, 1 M

20.3 g $MgCl_2 \cdot 6H_2O$
H_2O to 100 ml

$MgSO_4$, 1 M

24.6 g $MgSO_4 \cdot 7H_2O$
H_2O to 100 ml

MOPS buffer

0.2 M MOPS [3-(*N*-morpholino)-propanesulfonic acid], pH 7.0
0.5 M sodium acetate
0.01 M EDTA

Store in the dark and discard if it turns yellow

NaCl, 5 M

292 g NaCl
H_2O to 1 liter

NaOH, 10 M

Dissolve 400 g NaOH in 450 ml H_2O
Add H_2O to 1 liter

PBS (phosphate-buffered saline)

10× stock solution, 1 liter:
80 g NaCl
2 g KCl
11.5 g $Na_2HPO_4 \cdot 7H_2O$
2 g KH_2PO_4
Working solution, pH ~7.3:
137 mM NaCl
2.7 mM KCl
4.3 mM $Na_2HPO_4 \cdot 7H_2O$
1.4 mM KH_2PO_4

Potassium acetate buffer, 0.1 M

Solution A: 11.55 ml glacial acetic acid/liter (0.2 M).

Solution B: 19.6 g potassium acetate ($KC_2H_3O_2$)/liter (0.2 M).

Referring to Table A.2.2 for desired pH, mix the indicated volumes of solutions A and B, then dilute with H_2O to 100 ml.

This may be made as a 5- or 10-fold concentrate by scaling up the amount of potassium acetate in the same volume. Acetate buffers show concentration-dependent pH changes, so check concentrate pH by diluting an aliquot to the final concentration.

To prepare buffers with pH intermediate between the points listed in Table A.2.2, prepare closest higher pH, then titrate with solution A.

Potassium phosphate buffer, 0.1 M

Solution A: 27.2 g KH_2PO_4 per liter (0.2 M).

Solution B: 34.8 g K_2HPO_4 per liter (0.2 M).

Referring to Table A.2.3 for desired pH, mix the indicated volumes of solutions A and B, then dilute with H_2O to 200 ml.

This may be made as a 5- or 10-fold concentrate by scaling up the amount of potassium phosphate in the same volume. Phosphate buffers show concentration-dependent pH changes, so check concentrate pH by diluting an aliquot to the final concentration.

SDS electrophoresis buffer, 5×

15.1 g Tris base
72.0 g glycine
5.0 g SDS
H_2O to 1000 ml

Dilute to 1× or 2× for working solution, as appropriate

Store up to 1 month at 0° to 4°C

Do not adjust the pH of the stock solution, as the solution is pH 8.3 when diluted.

SED (standard enzyme diluent)

20 mM Tris·Cl, pH 7.5
500 ug/ml bovine serum albumin (Pentax Fraction V)

10 mM α-mercaptoethanol

Store at 4°C for up to 1 month

Sodium acetate, 3 M

Dissolve 408 g sodium acetate·$3H_2O$ in 800 ml H_2O
Add H_2O to 1 liter

Adjust pH to 4.8 or 5.2 (as desired) with 3 M acetic acid

Sodium acetate buffer, 0.1 M

Solution A: 11.55 ml glacial acetic acid/liter (0.2

M).
Solution B: 27.2 g sodium acetate ($NaC_2H_3O_2 \cdot 3H_2O$)/liter (0.2 M).

Referring to Table A.2.2 for desired pH, mix the indicated volumes of solutions A and B, then dilute with H_2O to 100 ml. (See Potassium acetate buffer recipe for further details.)

Sodium phosphate buffer, 0.1 M

Solution A: 27.6 g $NaH_2PO_4 \cdot H_2O$ per liter (0.2 M).
Solution B: 53.65 g $Na_2HPO_4 \cdot 7H_2O$ per liter (0.2 M).

Referring to Table A.2.3 for desired pH, mix the indicated volumes of solutions A and B, then dilute with H_2O to 200 ml. (See Potassium phosphate buffer recipe for further details.)

SSC (sodium chloride/sodium citrate), 20×

3 M NaCl (175 g/liter)
0.3 M $Na_3citrate \cdot 2H_2O$ (88 g/liter)
Adjust pH to 7.0 with 1 M HCl

STE buffer

10 mM Tris·Cl, pH 7.5
10 mM NaCl
1 mM EDTA, pH 8.0

TAE (Tris/acetate/EDTA) electrophoresis buffer

50× stock solution:
242 g Tris base
57.1 ml glacial acetic acid
37.2 g $Na_2EDTA \cdot 2H_2O$
H_2O to 1 liter
Working solution, pH ~8.5:
40 mM Tris·acetate
2 mM $Na_2EDTA \cdot 2H_2O$

TBE (Tris/borate/EDTA) electrophoresis buffer

10× stock solution, 1 liter:
108 g Tris base (890 mM)
55 g boric acid (890 mM)
40 ml 0.5 M EDTA, pH 8.0 (see recipe; 20 mM)

TE (Tris/EDTA) buffer

10 mM Tris·Cl, pH 7.4, 7.5, or 8.0 (or other pH;

see recipe)
1 mM EDTA, pH 8.0

TEA (triethanolamine) solution

50 mM triethanolamine, pH ~11.5
0.1% Triton X-100
0.15 M NaCl

Add Triton X-100 as a 10% stock sterilized by Millipore filtration and stored in the dark to prevent photooxidation (stock is stable 5 years at room temperature).

TEN (Tris/EDTA/NaCl) solution

40 mM Tris·Cl, pH 7.5 (*APPENDIX 2*)
1 mM EDTA, pH 8.0 (*APPENDIX 2*)
150 mM NaCl
Store up to 6 months at room temperature

TM buffer, 10×

100 mM Tris·Cl, pH 8.0
100 mM $MgCl_2$

Tris-buffered saline (TBS)

100 mM Tris·Cl, pH 7.5 (*APPENDIX 2*)
0.9% (w/v) NaCl (150 mM)
Store up to several months at 4°C

Tris·Cl [tris(hydroxymethyl)aminomethane], 1 M

Dissolve 121 g Tris base in 800 ml H_2O
Adjust to desired pH with concentrated HCl
Mix and add H_2O to 1 liter

Approximately 70 ml of HCl is needed to achieve a pH 7.4 solution, and approximately 42 ml for a solution that is pH 8.0.

NOTE: *The pH of Tris buffers changes significantly with temperature, decreasing approximately 0.028 pH units per 1°C. Tris-buffered solutions should be adjusted to the desired pH at the temperature at which they will be used. Because the pK_a of Tris is 8.08, Tris should not be used as a buffer below pH ~7.2 or above pH ~9.0.*

TTBS (Tween 20/TBS)

0.1% Tween 20 in Tris-buffered saline (TBS; see

recipe)
Store up to several months at 4°C

COMMENTARY

Background Information

There are a number of different procedures for the preparation of genomic DNA. They all start with some form of cell lysis, followed by deproteination and recovery of DNA. The main differences between various approaches lie in the extent of deproteination and in molecular weight of the DNA produced. The isolation procedure described here is relatively brief and relies on the powerful proteolytic activity of proteinase K combined with the denaturing ability of the ionic detergent SDS. Use of proteinase K for DNA purification was described by Gross-Bellard et al. (1973) and Enrietto et al. (1983). EDTA is included in the digestion buffer to inhibit DNases.

Critical Parameters

To minimize the activity of endogenous nucleases, it is essential to rapidly isolate, mince, and freeze tissue. Tissue culture cells should be cooled and washed quickly. As soon as the tissue is frozen or the tissue culture cells are added to the lysis buffer, DNA is protected from action of nucleases throughout this protocol. It is important that the tissue be well dispersed, and not left in large lumps, to permit rapid and efficient access to proteinase K and SDS.

It is crucial to generate high-molecular-weight DNA for construction of phage (>60 kb) or cosmid (>120 kb) genomic libraries. Two main precautions should be taken to maximize molecular weight: (1) minimize shearing forces by gentle (but thorough) mixing during extraction steps, and (2) after the extraction, remove organic solvents and salt from the DNA by dialysis, rather than by ethanol precipitation. Additional precautions must be taken to prepare very high-molecular-weight DNA for the construction of P1 or BAC libraries.

The absence of both cellular proteins and proteinase K in the final DNA solution is important for susceptibility of the genomic DNA to restriction enzyme action; therefore, care should be exercised in deproteination. To remove protein completely it may be necessary to repeat the proteinase K digestion. In general, highly pure DNA has an A_{260}/A_{280} ratio >1.8, while 50%

protein/50% DNA mixtures have A_{260}/A_{280} ratios of ~1.5.

Troubleshooting

Failure of the organic phase to separate cleanly from the aqueous phase is generally due to a very high concentration of DNA and/or cellular debris in the aqueous phase. Dilution with more digestion buffer and reextraction can remedy this problem.

Upon addition of the room-temperature ethanol to the extracted DNA solution, the DNA should precipitate in long, stringy fibers. If there is no precipitate or if the precipitate is flocculent, the DNA is either degraded or not purified away from cellular debris. Improper handling of the tissue before digestion or too much tissue in the digestion reaction are possible causes of such problems.

Anticipated Results

Approximately 2 mg DNA should be obtained from 1 g tissue or 10^9 cells. The DNA should be at least 100 kb long and should be digestible with restriction enzymes.

Time Considerations

This protocol involves effort on 2 days: tissue preparation on the first day followed by overnight lysis, and extraction/precipitation on the second day. Actual time spent on the procedure, however, will be less than 1 hr each day. The DNA can be stored indefinitely in the presence of ethanol at 4° or in TE buffer at -20°C.

Literature Cited

Enrietto, P.J., Payne, L.N., and Hayman, M.J. 1983. A recovered avian myelocytomatosis virus that induces lymphomas in chickens. Pathogenic properties and their molecular basis. *Cell* 35:369-379.

Gross-Bellard, M., Oudet, P., and Chambon, P. 1973. Isolation of high-molecular-weight DNA from mammalian cells. *Eur. J. Biochem.* 36:32-38.